

学位論文要旨

Development of an HPLC-fluorescence method for polycyclic aromatic hydrocarbons and their nitro-derivatives in atmospheric particulate matter and its application

大気粒子中の多環芳香族炭化水素及びそのニトロ誘導体の測定のための蛍光 HPLC 法の開発とその応用

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Abstract

An analytical method using high-performance liquid chromatography (HPLC) with fluorescence (FL) detection was developed for simultaneously analyzing 10 polycyclic aromatic hydrocarbons (PAHs) and 18 nitro-derivatives of PAHs (NPAHs). The two-dimensional HPLC system consists of an on-line clean-up and reduction for NPAHs in the 1st dimension, and separation of the PAHs and the reduced NPAHs and their FL detection in the 2nd dimension after column-switching. To identify an ideal clean-up column for removing sample matrix that may interfere with detection of the analytes, the characteristics of 8 reversed-phase columns were evaluated. The nitrophenylethyl (NPE)-bonded silica column was selected because of its shorter elution band and larger retention factors of the analytes due to strong dipole-dipole interactions. The amino-substituted PAHs (reduced NPAHs), PAHs and deuterated internal standards were separated on polymeric octadecyl-bonded silica (ODS) columns and by dual-channel detection within 120 min including clean-up and reduction steps. The limits of detection were 0.1 - 9.2 pg per injection for PAHs and 0.1 - 140 pg per injection for NPAHs. For validation, the method was applied to analyze crude extracts of fine particulate matter (PM_{2.5}) samples and achieved good analytical precision and accuracy. Moreover, the standard reference material (SRM1649b, urban dust) was analyzed by this method and the observed concentrations of PAHs and NPAHs were similar to those in previous reports. Thus, the method developed herein has the potential to become a standard HPLC-based method, especially for NPAHs.

Introduction

Polycyclic aromatic hydrocarbon (PAHs) and their nitro-derivatives (NPAHs) are well-known carcinogenic and/or mutagenic compounds in the atmosphere, especially dinitropyrenes such as 1,3-, 1,6- and 1,8-dinitropyrenes show strong direct-acting mutagenicity. Atmospheric PAHs and NPAHs are mainly originated from the incomplete combustion of organic materials such as coal, petroleum and biomass. Atmospheric NPAHs are produced not only by primary sources such as diesel engine exhaust, but also secondary reactions of their parent PAHs in the atmosphere. The toxic

PAHs and NPAHs are mostly distributed in airborne particulate matter (PM) and NPAHs are generally found at very low concentrations (several pg/m^3 ~ in total) which are about 1 - 3 orders of magnitude lower than their parent PAHs. Numerous researchers focused on only the 16 priority PAHs listed by U.S. Environmental Protection Agency (EPA). However, analysis of interrelationship between PAHs and NPAHs concentrations as well as individual component is very useful for source identification. For example, 1-NP is an important marker for automobile exhaust and the ratio of 1-NP to PAHs has been used to determine the contribution of car exhaust to urban PM samples. Several NPAHs formed in the atmosphere via reactions of their parent PAHs with OH or NO_3 radicals have been used as markers for atmospheric reactions. The analysis of both PAHs and NPAHs is essential to discuss sources and health effects of airborne PM such as $\text{PM}_{2.5}$.

Main study

In this study, an analytical method using HPLC with FL detection (HPLC-FL) was developed for simultaneously analyzing 10 PAHs and 18 NPAHs. The two-dimensional HPLC system consists of clean-up, reduction, column-switching, separation and FL detection steps. A schematic diagram of the HPLC-FL system used for the simultaneous determination of PAHs and NPAHs is shown in Fig. 1.

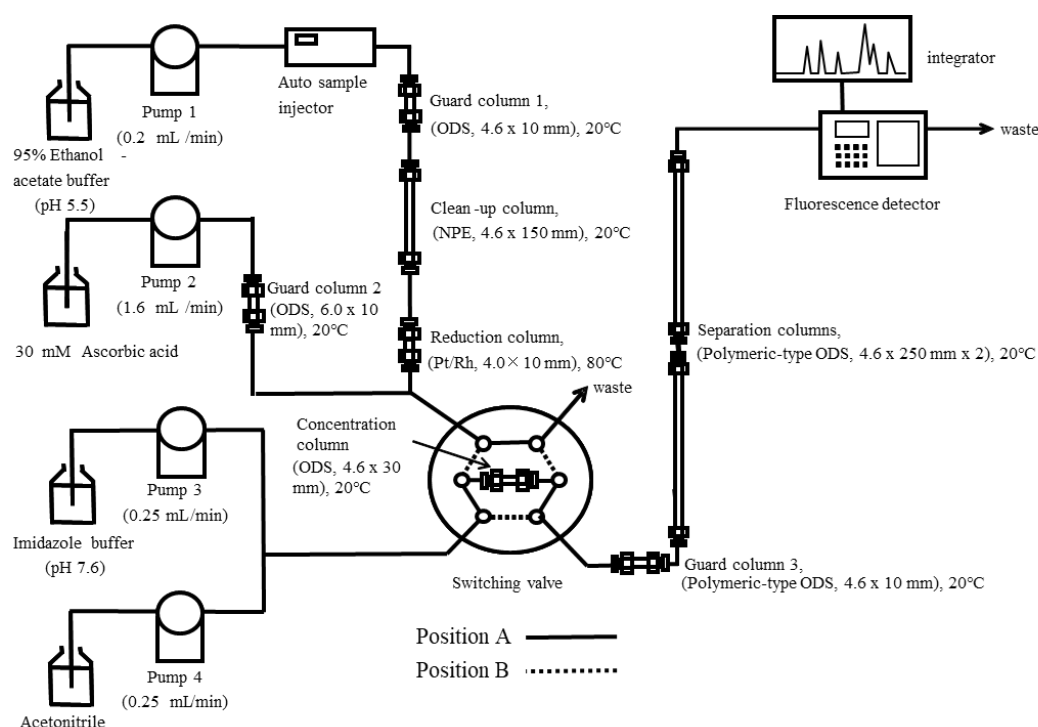


Fig. 1 Schematic diagram of the developed HPLC-FL method

To identify an ideal clean-up column for removing sample matrix that may interfere with detection of the analytes, the characteristics of 8 reversed-phase columns were evaluated. A high percentage of ethanol is necessary for the reduction step using a platinum/rhodium (Pt/Rh) column

and acetonitrile decreases the reduction efficiency. On the other hand, acetonitrile is an effective solvent for clear separation of all targets including similar isomers and to decrease column pressure. To eliminate opposing solvent effects, the two-dimensional system was able to switch solvent source to minimize the solvent effects of the 1st dimension. The clean-up column can be incorporated into the 1st dimension for a partial purification using column-switching. Since PAHs and NPAHs have a wide range of hydrophobicities, conventional ODS columns have little effects on removing substances that may interfere with the detection of analytes. The retention characteristics of 8 reversed-phase columns were evaluated to find a more effective column than the ODS column.

The ideal characteristics of a clean-up column are a short elution band and large retention factors (k) for all analytes. A shorter band can decrease the loading time to the concentration column and strong retention can increase the specificity of clean-up column. To satisfy these conditions, analytes with low $\log P$ values such as dinitropyrenes need to be retained with other interactions in addition to hydrophobic interaction, whereas the retention of the analytes with high $\log P$ values needs to be suppressed. PAHs and NPAHs were strongly retained on the tested columns having dipole-dipole and dispersive interactions and in addition to their hydrophobic and π - π interactions, though the hydrophobicity of the stationary phases is much smaller than that of ODS phases. An index, switching index, was used to evaluate the length of the elution band and the distribution of retention times. The switching index can be defined by Eq. (1);

$$\text{Switching index} = (k_{\text{max}} - k_{\text{min}}) / k_{\text{mean}} \quad (1)$$

where k_{max} , k_{min} and k_{mean} are the maximum, minimum and mean of retention factors (k) of all target compounds, respectively. A small value indicates a short elution band and strong retention of the analytes. Although the phenylethyl (PE)-bonded silica column showed the smallest switching index (0.93), the retention of the analytes on the column was considerably weaker than the other columns. Taking into consideration the separation of the analytes from a hydrophilic matrix, the nitrophenylethyl (NPE)-bonded silica column (switching index: 1.00) was selected as the clean-up column for the switching system. All the analytes were retained for over 15 min and eluted for 17.6 min (switching time: 17.4 - 33.0 min) under the optimal conditions for the clean-up and reduction columns.

After column switching, 10 PAHs and 18 amino-derivatives of NPAHs were separated on the separation columns which consist of 2 polymeric-type ODS columns (4.6 mm i.d. \times 250 each, 5 μm) in tandem and by dual-channel detection. Fig.2 shows typical chromatograms of a standard mixture of target PAHs, NPAHs and deuterated internal standards, which were all well separated by gradient elution. Total analytical time for simultaneously determining PAHs and NPAHs was 120.0 min including 33.0 min for clean-up and reduction steps in the 1st dimension. The analysis time for NPAHs was reduced by half compared to the conventional HPLC with chemiluminescence detection (HPLC-CL) and was followed by PAH analysis. Three deuterated PAHs and the amino-derivative of deuterated 6-nitrochrysene were separated from the non-deuterated compounds with sufficient resolution ($R_s > 2.85$). In general, stable isotope-labeled compounds are excellent internal standards

for mass spectrometric detection, but not for optical detection methods such as FL detection. However, deuterated PAHs can be separated from the non-deuterated analogues with baseline resolution on polymeric-type ODS columns and have nearly the same fluorescence characteristics. Dual-channel fluorescence detection was used to avoid frequent changes of the detection wavelengths or separate co-eluted or closely eluting peaks. The two-channel program was set based on optimal excitation and emission wavelengths of the analytes. Remarkably, 6-nitrochrysene and 7-nitrobenz[*a*]anthracene were successfully separated by their specific excitation and emission wavelengths, not by the separation columns as they had identical retention times (Fig.2). These results suggest that the proposed HPLC-FL system is suitable for the identification of all target compounds. The limits of detection were 0.1-9.2 pg per injection for PAHs and 0.1-140 pg per injection for NPAHs.

The LODs of PAHs and NPAHs were better than or comparable to those of the previous FL methods for PAHs and the CL methods for NPAHs. Calibration curves showed good linearity for all analytes ($r^2 > 0.9999$ for PAHs and > 0.99 for NPAHs). For validation, the method was applied to analyze crude extracts of fine particulate matter (PM_{2.5}) samples which were collected by a high-volume air sampler. The extraction method of PM samples involved ultrasonic extraction with dichloromethane, evaporation and redissolving steps, and then the crude extract was directly injected to the HPLC system.

The recoveries of the deuterated internal standards were 80 - 119% through the entire pretreatment. Known amounts of PAHs and NPAHs were added to PM_{2.5} samples, and their quantification accuracy and precision were evaluated. The accuracies were 86 - 98% and 87 - 109% for the PAHs and NPAHs, respectively. The precision was good with a RSD of 15% or less for all analytes. Both intra- and inter-day accuracy and precision were satisfactory for the simultaneous determination of all PAHs and NPAHs in the PM_{2.5} samples.

The concentrations of PAHs in the suburban PM_{2.5} samples used for the validation study were slightly higher than those in a background site located in the Noto peninsula without any major sources near the station. In contrast, the PAH levels were lower than samples collected at a road side in Kanazawa city and the other Japanese cities. PAHs are commonly found in PM_{2.5} fractions and PAH concentrations have been directly correlated with PM_{2.5} levels. The PAH concentrations in this study were reasonable, with respect to the PM_{2.5} levels of $7.7 \pm 2.9 \mu\text{g}/\text{m}^3$ measured during the sampling period. The concentration of 1-NP in this study was significantly lower than other sites, including the background site, indicating little emission source related to automobile exhaust around the site. The 1-NP contribution to NPAHs has substantially decreased throughout Japan in recent years, in conjunction with government regulations regarding automobile emissions. Moreover, the standard reference material of urban dust (SRM1649b) was analyzed by this method. The quantified PAH concentrations showed good agreement with the certified values in the range of 90 - 105%. Five NPAHs were highly consistent with the certified NPAH values (92 - 117%) and several compounds without their certified values showed similar concentrations with other literature data. There were

large variations in the reported concentrations of NPAHs from literature data, and these differences could arise from all of the procedure of the analytical protocols employed.

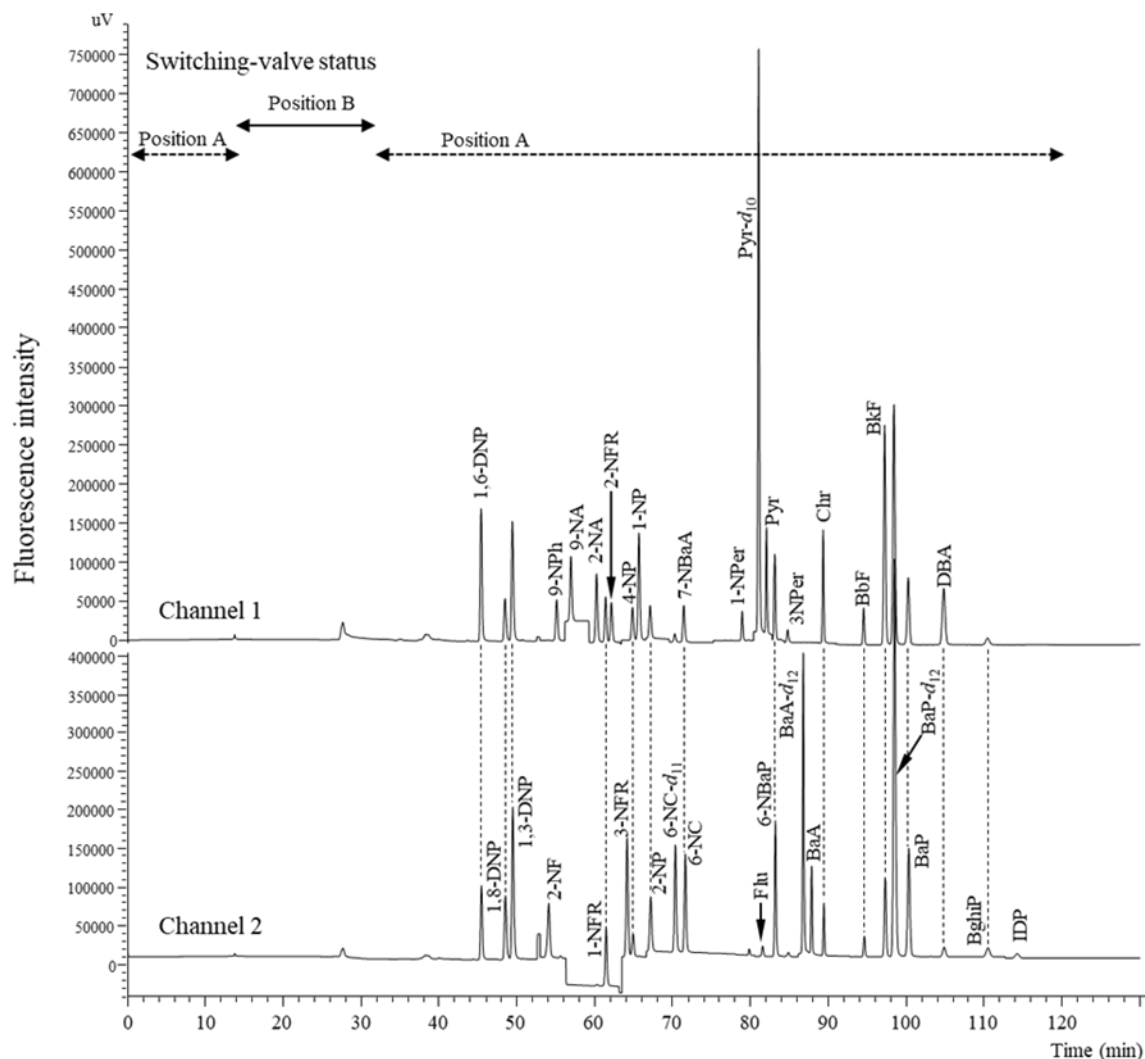


Fig.2 Representative standard chromatograms of PAHs and NPAHs measured by the developed HPLC-FL method. Injected amounts: Channel 1; 1,6-DNP, 750 pg; 9-NPh, 3 ng; 9-NA, 3 ng; 2-NA, 500 pg; 2-NFR, 1 ng; 4-NP, 12.5; 1-NP, 500 pg; 7-NBaA, 1 ng; 1-Nper, 10 ng; Pry-*d*₁₀, 11 ng; Pyr, 1 ng; 3-NPer, 10 ng; Chr, 1 ng; BbF, 1 ng; BkF, 1 ng; DBA, 1 ng; Channel 2; 1,8-DNP, 1 ng; 1,3-DNP, 1 ng; 2-NF, 500 pg; 1-NFR, 1 ng; 3-NFR, 5 ng; 2-NP, 4 ng; 6-NC-*d*₁₁, 6 ng; 6-NC, 1 ng; Flu, 1 ng; ; 6-NBaP, 6 ng; BaA-*d*₁₂, 6 ng; BaA, 1 ng; BaP-*d*₁₂, 7 ng; BaP, 1 ng; BghiP, 1 ng; IDP, 1 ng.

Conclusion

A two-dimensional HPLC-FL system has been developed for the simultaneous determination of 10 PAHs and 18 NPAHs. The column switching system enables direct injection of crude PM sample extract without complicated pretreatment. The NPE column as the clean-up column showed

short elution band and strong retention of the analytes by dipole-dipole interactions and displayed effective fractionation of the analytes from the sample matrix in the 1st dimension. Good separation of all target compounds was achieved by polymeric-type ODS columns and dual-channel FL detection in the second dimension. The time required for analysis of NPAHs was cut in half when compared to conventional CL methods with a total time of 120 min, including analysis of PAHs. The LODs of PAHs and NPAHs were better than or comparable to those of the previous FL methods for PAHs and the CL methods for NPAHs. The FL detection can reduce solvent consumption and eliminate the requirement for pumps for the post-label reagents for the CL detection of NPAHs. This HPLC-based method also enables much larger-scale injections (100 μ L or more) compared to GC-based methods, therefore, it is applicable for samples with a small sampling volume limited by collecting instruments such as personal samplers. The quantification of PAHs and NPAHs in crude extracts of PM_{2.5} samples showed good accuracy and precision and concentration determinations for SRM 1649b were similar to the certificate and other values reported in the literature. Considering the above-mentioned advantages, the developed method has the potential to a standard HPLC-based method, especially for NPAH determination.

審査結果の要旨

〔審査経過〕平成 29 年 7 月 29 日に、審査方針に従って口頭発表（最終試験）を行い、終了後に開催した論文審査委員会において協議の結果、以下のように判定した。

〔審査結果〕主要な大気汚染物質の 1 つである微小粒子状物質(PM_{2.5})には、発がん性／変異原性を有する多環芳香族炭化水素（PAH）やそのニトロ誘導体（NPAH）が含まれており、それらの大気モニタリングが重要視されている。PAH や NPAH は、ガスクロマトグラフや高速液体クロマトグラフ（HPLC）に様々な検出器を組み合わせで測定されてきたが、1 回の分析で同時に測定できる分析法はほとんど報告されていない。本研究では、10 種の PAH と 18 種の NPAH を同時に分析できる、蛍光検出・HPLC による高感度な分析法を開発した。試料の精製と NPAH の還元を行う部分と分離・検出を行う部分から成る二次元 HPLC システムとすることで大気試料の粗抽出物の直接分析が可能となった。さらに開発した分析法を実試料に適用した結果、良好な分析精度および精度を達成した。本研究により開発された分析法は、今後 PAH 及び NPAH のための標準的な HPLC による分析法になり得ると考えられる。以上の成果より、審査委員会は学位申請論文が博士（学術）に値すると判定した。